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(54) Title: METHODS AND CLINICAL DEVICES FOR THE INHIBITION OR PREVENTION OF MAMMALIAN CELL GROWTH

(57) Abstract: Mammalian cell growth may be inhibited by use of a chemical material containing at least one biguanide group -NH-C(NH)-NH-C(NH)-NH- in the preparation of a medicament or medical device for the inhibition of mammalian cell growth. The chemical material may be monomeric or polymeric.

WO 02/064127 A1

**METHODS AND CLINICAL DEVICES FOR THE INHIBITION OR
PREVENTION OF MAMMALIAN CELL GROWTH**

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FIELD OF THE INVENTION

This invention relates to the inhibition of cell growth, more particularly it relates to the inhibition of mammalian cell growth. In one embodiment it relates to the preparation of medical compositions for treatments for the inhibition of mammalian cell growth. In another embodiment it relates to the inhibition of mammalian cell growth where tissue growth is a problem, e.g. in the case of cancerous growths, or in the vicinity of implanted clinical devices. Still more particularly it relates to the prevention or inhibition of restenosis for articles such as stents implanted in blood vessels after angioplasty. More specifically, the invention concerns the use of monomeric or polymeric biguanides to inhibit mammalian cell growth. In one particular embodiment, it relates to the use of a family of polymeric materials incorporating biguanide compounds pendant to the polymer chain in the production of articles of manufacture in particular medical devices.

20

Biguanides are a family of chemical compounds that contain a grouping of the formula:



the terminal nitrogen atoms being attached to any of a broad range of groups, and many of them have previously been identified as having powerful antimicrobial activity. Their action on mammalian cells, however, has been far less intensively investigated.

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BACKGROUND OF THE INVENTION

Under normal conditions, cell growth occurs throughout the life of the organism. Usually this proceeds in the manner that is normal for the particular part of the body of that

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organism, but occasionally in specific instances such as healing of tissues, additional growth is needed until the tissue is repaired, at which time it should cease, and normal functioning be resumed. Occasionally, however, the normal growth-inhibiting factors cease to operate and cell growth continues unchecked.

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There are several clinical areas in which mammalian cell growth poses a problem. Atherosclerosis, a major cause of death in the western world, is commonly treated by the operation of angioplasty. This produces the immediate effect of dilation of the blood vessel, but there is a continuing problem of restenosis, i.e. narrowing of the blood vessel, because of the proliferation of smooth muscle cell, and the deposition of extracellular matrix proteins such as collagen.

10

Similar problems resulting from undesired cell growth may be encountered after other surgical treatments, such as the installation of prostheses, implanted sensors, or implanted capsules that contain cells or release drugs.

15

There are, moreover, several medical conditions in which undesired growth of cells occurs, sometimes with results that are disfiguring, as in the case of the "Elephant Man", who suffered a number of highly visible growths of tissue. Previously thought to be a case of neurofibromatosis, it is now thought to be a very rare condition, known as the Proteus syndrome.

20

Where restenosis is concerned, because of the scale of the problem arising from the number of angioplasties now carried out with an aging population, and with the tendency among western peoples to adopt an unsatisfactory diet, there have been intensive efforts to alleviate restenosis, see the review article "Restenosis - an open file" by Gottsauner-Wolf *et al.* in Clin. Cardiol. 19, 347-356 (1996). A wide variety of pharmaceuticals have been studied, and some success has been achieved with platelet inhibitors, glycoprotein IIb/IIIa receptor antagonists, and the use of intracoronary metallic stents. There is still, however, a need for effective means of alleviating the problem of stenosis.

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It is clear, therefore, that there are many medical fields in which a simple means of inhibiting undesired cell growth is urgently needed.

One object of this invention is to provide a solution to this problem.

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Another object of this invention is to provide compositions that can be administered to a patient orally, or by injection, or by implantation.

Another object of this invention is to provide compositions that can be applied to surgical
10 devices, including but not limited to stents, stent grafts, vascular grafts and surgical prostheses, to inhibit restenosis and other undesired cell growth.

Another object of this invention is to provide compositions that can be used to manufacture surgical devices, including but not limited to stents, stent grafts, vascular
15 grafts and surgical prostheses, to inhibit restenosis and other undesired cell growth.

Another object of the invention is to provide compositions that comprise or can be used in conjunction with the biguanide that also contain anti-cancer agents, antibiotic agents and/or drugs. The purpose of these compositions is to provide an action of the biguanide
20 that facilitates entry of the agent or drug through the cell wall. The object is to allow lower dosage of the agent or drug, thereby reducing the side effects of the agent or drug.

The agent or drug may be eluted from a medical device whereas the biguanide may be bound to or associated with especially the surface of the medical device or may also be
25 eluted with the agent or drug. Such agents or drugs may be, but are not restricted to, rapamycin, actinomycin, and taxol.

Our prior British Patent Application GB-A-2349644, and corresponding International Patent Application PCT/GB00/0164, disclose polymeric materials incorporating a
30 biguanide compound pendant to the polymer chain, and chemically bonded thereto

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through some but not all of the amine nitrogen atoms of the biguanide compound. The present Application incorporates by reference these applications.

According to one embodiment, the present invention provides a method of inhibiting
5 undesired cell growth by bringing cells that may proliferate undesirably into effective exposure to a composition comprising at least one biguanide.

According to another embodiment, the present invention provides a method of providing
a medical device with the property of inhibiting undesired cell growth, by manufacturing
10 said device with a composition comprising at least one biguanide, adapted to bring cells that may proliferate undesirably into effective exposure to said biguanide. This may be achieved by employing a composition that will provide a permanent covalent linking of the biguanide to the device. Alternatively, the biguanide may be included in a water-insoluble composition deposited on the medical device, or a high-molecular weight
15 composition such as a polymer biguanide, may be entrapped or adsorbed on the medical device. Another method is to complex a biguanide with a metal. In general, the biguanide, either in monomeric or polymeric form, can be applied to the medical device from an organic or aqueous system or from water. The biguanide in monomeric or polymeric form can be caused to bind to the medical device by absorption, adsorption,
20 ionic interaction, or covalent attachment. Alternatively the biguanide need not be attached to the medical device but may be released close to the device or to the portion of the patient's anatomy that is to be protected from undesired cell growth by any conventional drug delivery means, e.g. polymer, liposomes, balloon catheters, etc.

25 According to another embodiment, the present invention relates to the use of a composition comprising at least one biguanide in a method of providing a medical device with the property of inhibiting undesired cell growth, in the manufacture of said device whereby, in use, said device will be capable of bringing cells that may proliferate undesirably into effective exposure to said biguanide.

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According to another embodiment, the present invention relates to the use of a composition containing at least one biguanide group together with at least one additional material having a physiological or psychotropic action,. Such compositions may be used either for direct administration, or may be applied to medical devices, in the same manner
5 as the compositions described above.

- Generally, this invention is directed to inhibiting, fully or partially, cellular encroachment, restenosis, and the like, by exposing cells to biguanides. One way in which this may be done in accordance with the invention is to associate the biguanide with an implant.
- 10 Generally the biguanides may be bound to a surface or released. Binding to a surface may be achieved by numerous methods including, but not limited to, adsorption, mixing, covalent bonding, ionic interaction, coulombic interaction, hydrogen bonding, interpenetrating networks, or crosslinking.
- 15 The release of biguanides may be accomplished by the use of controlled release mechanisms, mechanisms that rely on a coating, multiple coatings, microspheres, liposomes, pumps, gels, mixes, coacervates, leaching, dissolving, eroding, cell-mediated erosion, etc. The biguanides are a large class of compounds, and are useful in accordance with the present invention because of their effect on cells similar to that of chlorhexidine
20 and polyhexanide. The biguanides may be used in admixture with pharmacologically active and/or inert substances.

The biguanides used in accordance with the invention may be coated on, or directly attached to, materials used in the manufacture of medical devices, such as polymers,
25 metals, or ceramic materials for stents, stent grafts, vascular grafts and surgical prostheses.

The biguanides used in accordance with the invention may be used in conjunction with one or more additional materials having a physiological or psychotropic action.

Fabrication of medical apparatus is usually from metals or polymeric materials that may comprise, but are not limited to, polyurethanes, silicones, polyvinylchloride and others, by moulding and extrusion techniques.

- 5 Although many attempts have been made to eradicate the problem of infection on medical apparatus, we are presently unaware of any proposals to provide such apparatus with a means of inhibiting mammalian cell growth. Documents that disclose the attachment of an infection resistant material to the polymeric apparatus include US Patents No. 3,695,921; 4,581,028; 4,479,795; 2,084,466; 4,713,402; 4,678,660; and 5,451,424.

10

A number of documents disclose the use of various biguanides for providing medical devices with antiseptic properties.

- 15 Japanese Patent Application No 60/36064 discloses the adsorption of chlorhexidine on to the surface of polyurethane or silicone catheter by dipping into an aqueous solution of chlorhexidine which is then converted into a water insoluble form by dipping into a solution of an acid.

- 20 Japanese Patent Application No 59/228,856 discloses an elastomeric catheter possessing a water insoluble biguanide or salt as a thin coating on the surface.

PCT Application No WO 86/02561 discloses a thermoplastic polymer having up to 1% chlorhexidine contained in or upon the surface.

- 25 UK Patent Application No 2084466A discloses a procedure for rendering polypropylene apparatus biocidal with chlorhexidine base, and suggests that the apparatus may be prepared from other plastics.

- 30 US Patent No 4,891,423 discloses linear polyoxyalkylene diamine biguanides and discusses other known biguanides, and their use in solid and liquid bactericidal and fungicidal compositions, including ophthalmic saline solutions.

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US Patent No 5,142,010 discloses the vinyl copolymerisation of certain polymerisable unsymmetrical biguanide compounds.

US Patent No 5,817,325 discloses crosslinking biguanide polymers with, *inter alia*,
5 isocyanates or epoxides to form an immobile, insoluble, non-leachable surface matrix which has the ability to deliver deposited biocidal silver salts into a the interior of a micro-organism. The polymers are useful for coating contact lens cases and other articles. The biocidal action is through the silver salts, not through the highly cross-linked biguanides.

10 SUMMARY OF THE INVENTION

In accordance with the invention, novel chemically modified mammalian cell growth inhibiting materials are produced by the chemical modification of biguanide compounds to produce polymers that can be blended into the bulk of other polymers, be used as
15 coatings or in deposits, or be chemically attached to the surface of, or otherwise associated with, a medical device.

A medical device in accordance with the invention may be made of, coated with, or surface treated to form *in situ*, the novel polymers having pendant biguanide groups.

20 -NH-C(NH)-NH-C(NH)-NH-
have previously been identified as having powerful antimicrobial activity.

Biguanides are strongly basic compounds containing the biguanide group

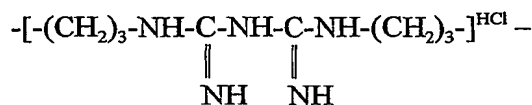
-NH-C(NH)-NH-C(NH)-NH-

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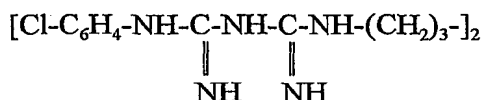
Two particularly preferred biguanides for the purposes of the present invention are polyhexanide and chlorhexidine, which are commercially available. Each includes the biguanide group adjacent a hexamethylene chain. Their respective structures can be represented as follows:

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Polyhexanide hydrochloride salt

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Chlorhexidine

Polyhexanide and chlorhexidine have a broad spectrum of anti-bacterial activity and at relatively low concentrations the anti-bacterial action is bacteriostatic; at higher
 15 concentration the action becomes rapidly bactericidal. The commercial water soluble salt of chlorhexidine is usually the digluconate.

In one aspect of the invention, biguanides are chemically modified to yield novel chemically modified materials that inhibit mammalian cell growth.

20

In another aspect of the present invention the chemically modified material is applied to medical devices to render them inhibitory of mammalian cell growth.

In accordance with a further aspect of this invention, the application of the chemically
 25 modified material to a medical device produces stable, non-leaching material that is inhibitory of mammalian cell growth.

Without wishing to be bound by any particular theory, it is believed that the mammalian cell growth inhibitory activity of biguanide compounds derives from the strongly basic
 30 character of their biguanide groups which form acid addition salts with a cationic charge delocalised over the five neighbouring secondary amine nitrogen atoms. This enables the biguanide to be rapidly attracted to a negatively charged cell. Thereafter the biguanide

interacts with the cytoplasmic membrane, upsetting the ionic balances and, ultimately, disrupting the membrane and causing irreversible damage to the cell contents.

The present invention utilises the amino nitrogen atoms of the biguanide group to anchor
5 these mammalian cell growth inhibitory compounds to a polymeric substrate as pendant species without disabling their mammalian cell growth inhibitory properties, although the normal acid addition salt form of these compounds interferes with their derivatisation at these amino sites. The polymeric products of the invention are thus distinguished from both linear polymeric biguanides and highly cross-linked biguanide polymers.

10

In accordance with one embodiment of the invention, there is provided a polymeric material incorporating a mammalian cell growth inhibitory biguanide compound pendant to the polymer chain, being chemically bound thereto through some but not all of the amine nitrogen atoms, and preferably of the secondary amine nitrogen atoms of the
15 -NH-C(NH)-NH-C(NH)-NH- biguanide group or groups, of the inhibitory biguanide compound. The pendant biguanide compounds are generally bound through the secondary amine nitrogen atoms, which may include some of the >C=NH imino nitrogen atoms, and may include some of the C-NH-C secondary amine nitrogen atoms. However, a certain amount of binding through the primary amine groups at each end of biguanides
20 such as polyhexanide is also possible.

There is also provided a medical device comprising a polymeric material incorporating a pendant mammalian cell growth inhibitory biguanide compound chemically bound to the polymer through some but not all of the amine nitrogen atoms of the biguanide, and
25 particularly the secondary amine nitrogen atoms of the -NH-C(NH)-NH-C(NH)-NH- biguanide group or groups of the biguanide compound. Such a medical device may be formed from or coated with the polymeric material incorporating the biguanide compound, or the medical device may first be formed from or coated with polymeric material which is thereafter chemically bound to some but not all of the nitrogen atoms of
30 the biguanide compound, or the medical device may first be formed from or coated with polymeric material which is thereafter chemically bound to the residuum of a non-

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polymeric compound that has been bound to some but not all of the nitrogen atoms of the biguanide compound.

In other words, the biguanide can be incorporated as a pendant group into a polymer
5 which is then made into or coated on to an article, or the biguanide can be chemically linked to polymer already on an article, or the biguanide can be bound to polymer on an article through an intermediate non-polymeric compound. Such a compound requires one functionality to bind with the biguanide secondary amine, and one functionality to bind with the polymer.

10

Polymer functionality to bind with the material inhibiting mammalian cell growth directly (either to the biguanide secondary amine or to a functional group on a bound non-polymeric compound as described above) may include groups such as hydroxyl (-OH), carboxyl (-COOH), anhydride (-CO-O-CO-), isocyanate (-NCO), allyl, vinyl, acrylate,
15 methacrylate, epoxide, sulfonic (-SO₃⁻) or sulfate (-SO₄⁻) groups. Linkage to the polymer may be by covalent bonding (including grafting) or by ionic bonding.

Chemical binding to a secondary amine nitrogen atom by means of isocyanate results in a substituted urea linkage, or by means of isothiocyanate results in a substituted thiourea
20 linkage, or by means of epoxide results in a beta-hydroxyl-tertiary amine, or by means of acid chloride results in a N,N-disubstituted amide, or by means of acid anhydride results in a N,N-disubstituted amide, or by means of aldehyde or ketone results in N,N-disubstituted hemiaminals or amins depending on the aldehyde or ketone, or by means of unsaturated bonds results in a tertiary amine linkage.

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Suitable medical devices to which the invention may be applied include catheters, vascular grafts, stents, stent grafts, heart valves and pumps.

In a further aspect, the invention provides a method of making polymeric material which
30 comprises chemically binding reactive sites on a polymeric material with some but not all of the amine nitrogen atoms of a biguanide compound, especially the secondary amine

nitrogen atoms of the -NH-C(NH)-NH-C(NH)-NH- biguanide group or groups of a biguanide compound. The secondary amine nitrogen atoms bound to the reactive sites may include some of the >C=NH imino nitrogen atoms and may include some of the C-NH-C nitrogen atoms. Primary amine end groups on suitable biguanide compounds, such as polyhexanide, may also participate in binding to polymeric materials.

In an important aspect of the method, it comprises the preliminary step of forming a free base, preferably a partial free base, of the biguanide compound before binding the reactive sites with the nitrogen atoms. By removing some but not all of the acid of the usual acid addition salt, some of the secondary amine nitrogen atoms become available for derivatisation. If the entire free base is liberated, care needs to be taken to ensure only partial derivatisation.

The preferred reactive sites to bind with the biguanide nitrogen comprise isocyanate, isothiocyanate, epoxide, acid chloride, acid anhydride, aldehyde, ketone and unsaturated (especially acrylate, methacrylate and vinyl) sites.

Similar considerations apply to a variation of the above method which comprises modifying a polymer precursor by chemically binding some but not all of the amine nitrogen atoms of a biguanide compound, especially the secondary amine nitrogen atoms of the -NH-C(NH)-NH-C(NH)-NH- biguanide group or groups of a biguanide compound with reactive sites on the polymer precursor, and thereafter converting the so modified polymer precursor to a polymeric material by a method including a polymerisation step which leaves the biguanide compound residue pendant to the polymer chain.

25

The reactive sites on the polymer precursor may comprise isocyanate, isothiocyanate, epoxide, acid chloride, acid anhydride, aldehyde, ketone or unsaturated sites, or other suitable sites. Even sites comprising hydroxyl, carboxyl or amino groups can link on to the biguanide groups by using coupling agents such as carbonyl diimidazole or carbodiimides.

30

The polymer precursor may also contain acrylate, methacrylate, allyl or vinyl groups, and the polymerisation step may be carried out by polymerising the modified polymer precursor through the said groups. Any other polymerisable group may also be used.

- 5 Similar considerations apply to a further variation of the foregoing method of making polymeric material that inhibits mammalian cell growth which comprises modifying a non-polymeric compound by chemically binding some but not all of the amine nitrogen atoms of a biguanide compound, especially the secondary amine nitrogen atoms of the -NH-C(NH)-NH-C(NH)-NH- biguanide group or groups of a biguanide compound with
- 10 reactive sites on the non-polymeric compound, and thereafter chemically binding the so modified compound to a polymeric material. The chemical binding of the material inhibiting mammalian cell growth to the polymeric material may be by covalent bonding (including grafting) or by ionic bonding.
- 15 The non-polymeric compound may also contain acrylate, methacrylate, allyl or vinyl groups, so that the modified compound may be chemically bound to a polymeric material through the said groups. Any other polymerisable group may also be used. Other functional groups carried by the non-polymeric compounds for binding with polymeric materials may include hydroxyl, carboxyl, amide, amino, epoxide, isocyanate, sulfate,
- 20 sulfonate and others. In general, it is possible to provide functionality that can react with available complementary chemical constituents contained in polymeric materials to form polymeric mammalian cell growth inhibitory materials or to covalently attach to surfaces.

- Whichever method of preparation is used, the resulting polymer containing biguanide
- 25 groups may be subsequently blended with other polymeric material to form a polymer blend for use in forming an article of manufacture, and preferably blended with medically acceptable polymeric material to form a polymer blend for use in the manufacture of a medical device.

- 30 Typical materials for blending include polyurethanes, polyamides, latex, silicones, siloxanes, polyvinyl chloride, polyesters, polycarbonates, polyacrylonitrile,

polymethylmethacrylate, polypropylene, polyethylene and hydrogels. Clearly the biguanide polymer and the blending polymer should be suitably compatible.

In a further application of the invention, the polymer containing biguanide groups may
5 subsequently be coated on to an article of manufacture to form a mammalian cell growth inhibitory coating thereon.

Chlorhexidine and polyhexanide are the preferred biguanide compounds. Each has its own activity spectrum. To broaden the mammalian cell growth inhibitory range of the
10 end product, the invention includes the further step of blending the resulting polymers containing biguanide groups derived from both chlorhexidine and polyhexanide, or copolymerising precursors to form a polymer in accordance with the invention containing biguanide groups derived from both chlorhexidine and polyhexanide.

15 The mammalian cell growth inhibiting material may exist in the free base or acid form or the salt thereof and as such the invention does include such forms.

DETAILED DESCRIPTION OF THE INVENTION

20 To illustrate the invention by a general example, an amino constituent contained in the mammalian cell growth inhibiting material (MCGIM) is able to react with a polymeric isocyanate (P-NCO) constituent to form a urea linkage giving a polymeric infection resistant material, as outlined in Scheme 1 a), b) and c). The converse is also true where the MCGIM contains isocyanate constituents and is able to react with polymeric amino.

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Scheme 1



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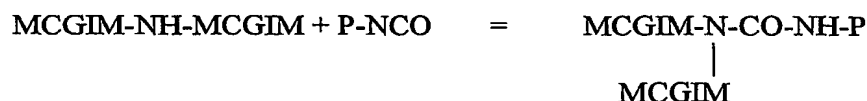
Polymeric Materials (PMCGIM) prepared as in Scheme 1 may be constituted, by way of example, into homogeneous blends of extrudable polyurethane to form medical apparatus
 5 with mammalian cell growth inhibiting properties that are effective and stable, in which the PMCGIM is unable to permeate to body tissue or fluids.

By way of another example the PMCGIM is prepared and dissolved in a suitable solvent for coating the medical article in order to give it mammalian cell growth inhibiting
 10 properties that are effective and stable, in which the PMCGIM is unable to permeate to body tissue or fluids.

By way of a further example the chemically modified MCGIM (e.g. MCGIM-NH₂) is attached to the surface by conventional chemical linkages. In the case of MCGIM-NH₂,
 15 one such method would be by way of an amide bond.

An example of chemical modification, in relation to polyhexanide, is the reaction of an isocyanate to the secondary amine, as shown by Scheme 2, to give substituted ureas.

20 Scheme 2

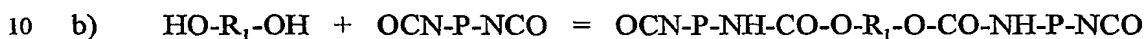
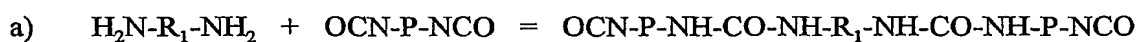


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The polyisocyanates useful in the invention in producing substituted ureas with the MCGIM, typically polyhexanide, may be selected from a wide range of aliphatic, cycloaliphatic, and aromatic polyisocyanates. The isocyanate groups may be carried on polymers having unsaturated alkyl groups, esters, ethers, siloxanes, urethanes, amides,
 30 carbonates, and mixtures thereof which can be chosen to promote compatibility with other polymers that they may subsequently be coated on or blended with.

Polydiisocyanates that can be utilised are those typically used in the formation of polyurethane, which when reacted with secondary amines form the substituted ureas. Additionally, polydiisocyanates can be prepared by the reaction of a polyamine or polyol with a diisocyanate, as shown by Scheme 3.

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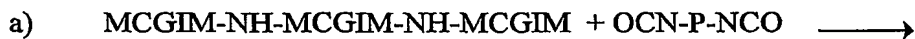
Scheme 3

In Scheme 3, -R₁- and -P- can typically be aliphatic groups, cycloaliphatic groups, aromatic groups, unsaturated alkyl groups, esters, ethers, siloxanes, urethanes, amides, carbonates, and mixtures thereof. Others are of course possible.

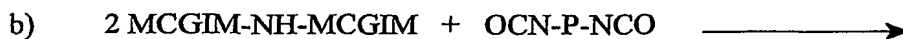
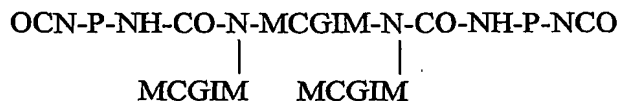
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Such polydiisocyanates may then be further reacted with MCGIM containing appropriate reactive chemical groups, an example being the secondary amine of polyhexanide reacted with the isocyanates, as shown in Scheme 4; thus producing Polymeric MCGIM (PMCGIM).

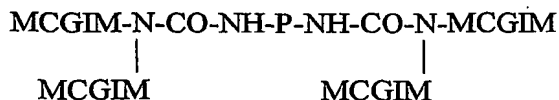
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Scheme 4

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The PMCGIM can be compounded with other polymers, such as polyurethanes, polysiloxanes, polyesters, polyvinylchlorides, polybutadienes and polyamides, to produce medical apparatus, either by extrusion or moulding, that is mammalian cell growth inhibiting. The active ingredient (MCGIM) is stable within the medical apparatus and is
5 non-leaching.

The PMCGIM or the MCGIM may contain allyl, vinyl, acrylate or methacrylate groups for polymerisation to form allyl, vinyl, acrylate, and methacrylate type polymers.

10 Allyl, vinyl, acrylate and methacrylate functionalities can be incorporated in the MCGIM by reacting the acid chloride, isocyanate, epoxide or anhydride of a molecule containing the above double bond functionality.

For instance, methacryloylchloride can be reacted with the secondary amine of a
15 biguanide resulting in the formation of a tertiary amide with the liberation of hydrogen chloride which re-forms the hydrochloride on the biguanide group.

Isocyanatoethyl methacrylate, allyl isocyanate, glycidyl methacrylate and the anhydride or mixed anhydride of methacrylic acid can undergo reactions with the free base of the
20 biguanide to yield methacrylate and allyl functionality on the biguanide group. The isocyanate would react to form a urethane urea bond, the epoxide would react to form a tertiary amine and the anhydride to form a tertiary amide.

Methacrolein can also react with the secondary amine of the biguanide. Here the reaction
25 is between a secondary amine and an aldehyde which can yield a hemiaminal or aminal, depending on the aldehyde.

Allyl, vinyl, acrylate and methacrylate derivatives of MCGIMs can undergo homopolymerisation or copolymerisation with numerous other molecules or polymers
30 which have a double bond under thermal or electromagnetic radiation. The allyl, vinyl, acrylate and methacrylate derivatives of MCGIMs can be grafted on to surfaces having

functional groups, eg OH, COOH, SO_3^- , SO_4^- , NH_2 , by using initiators such as ferric ammonium nitrate.

Conversely the free base of the biguanide can react with acrylate and methacrylate
5 derivatives of monomers or polymers to produce PMCGIM.

The PMCGIM may be dissolved in a suitable solvent, such as alcohols, acetone or tetrahydrofuran (THF) or mixtures thereof and coated on to medical apparatus. Dipping, spraying, or any other means by which a homogenous coating may be obtained, following
10 by any necessary drying out, can be used to place the coating of PMCGIM on to the medical apparatus. The articles to be coated may be made of plastics, metals, composites or any other material compatible with the intended coating.

The chemical materials containing at least one biguanide group may also be employed in
15 conjunction with one or more additional materials having a physiological or psychotropic action. The biguanide facilitate the passage of the material having a physiological or psychotropic action through the cell wall, thus allowing that material to be employed in lower dosages, thereby reducing potential side effects.

20 Instances of the manner in which such compositions may be employed include parenteral methods, e.g. tablets, capsules, emulsions, suspensions, or solutions; by injection, e.g. by needles or through drips; or by implantation, e.g. through degradable or non-degradable polymer carriers, or through pumps, e.g. infusion, peristalsis or osmosis.

25 Non-limiting examples of physiologically or psychotropically-active materials include:- materials used in chemotherapy, for cancer or other conditions, including adriamycin, alkeran, Ara-C, BiCNU, Busulfan, CCNU, Carboplatinum, Cisplatinum, cytoxan, Daunorubicin, DTIC, 5-FU, fludarabine, Hydrea, Idarubicin, Ifosfamide, Methotrexate, Mithranmycin, Mitomycin, mitoxantrone, nitrogen mustard, Taxol, Velban, Vincristine,
30 VP-16, Gemcitabine (Gemzar), Herceptin, Irinotecan (camptosar, CPT-11), Leustatin,

Navelbine, Rituxan, STI-571, Taxotere, Topotecan (Hycamtin), Xeloda (Capacitabine), and Paclitaxel.;

materials used for the prevention of bone metastasis or the treatment of high calcium
5 levels, such as Zometa (zoledronic acid);

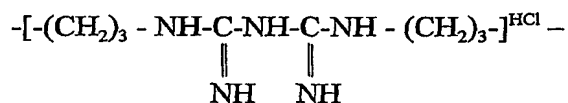
materials used for the treatment of low white blood cell counts, such as Peg-Filgrastim;
materials used for the treatment of anaemia, such as NESP; and

10 antibiotics, such as Amoxicillin, Ampicillin, aminoglycosides, cefaxolin, cefepime, sirolimus, actinomycin, vancomycin.

The invention is illustrated by the following non-limiting examples. Examples 1 and 2
concern the preparation of polyhexanide partial free-base, which is necessary in order to
15 protect some of the biologically active sites while freeing others to participate in
reactions, in order to prepare active infection-resistant derivatives.

Polyhexanide is a commercial anti-microbial agent manufactured by Zeneca Biocides and
can be represented by the following general formula:

20



25



One polyhexanide biguanide repeating unit

M.W. 219 ; where the average n = 12

In order to derivatise polyhexanide, at least some of the hydrochloride groups must be
30 removed. The hydrochlorides can be neutralised with a strong base, for example, sodium
hydroxide.

- Either all the hydrochloride groups associated with the biguanide groups of the polyhexanide can be neutralised and then the desired chemistry carried out before re-forming their hydrochloride salts or only the desired number of hydrochlorides can be neutralised and then the chemistry carried out before re-forming their hydrochlorides.
- 5 Both are acceptable methods of derivatisation.

Similar considerations apply to chlorhexidine and other biguanide compounds.

- Various methods of producing polymers linked to biguanides are described in the following non-limiting Examples. These polymers may be employed in the production of medical devices that are resistant to mammalian cell growth, by using any of the production techniques described in our prior Applications mentioned above.
- 10

Example 1

Polyhexanide starting material

15

- 400ml of a 20% w/v aqueous solution of polyhexanide (Zeneca Biocides) was placed in a Spectra/Por® membrane (MWCO: 2,000) and was dialysed against 10 litres of deionised water for 16hrs. The dialysed polyhexanide was then placed in stainless steel freeze-drying trays and was freeze dried for 72hrs.
- 20

The yield of dry crystalline powder of polyhexanide was 40g.

Example 2

Partial free-base polyhexanide

25

In this example only 1 in 6 biguanide hydrochloride groups are neutralised.

- 1g (4.5662×10^{-3} moles of biguanide hydrochloride groups) of polyhexanide powder (from Example 1) was dissolved in 80ml deionised water. The number of moles of sodium hydroxide required to neutralise 1 in 6 biguanide hydrochloride groups of polyhexanide is
- 30

-20-

7.61 x 10⁻⁴ moles (0.0304g, NaOH). Sodium hydroxide (0.0304g) was dissolved in 50ml deionised water and added drop-wise to the polyhexanide solution over a period of 1hr. The solution was then freeze-dried yielding a dry crystalline powder of polyhexanide partial free-base (Ph.P free-base).

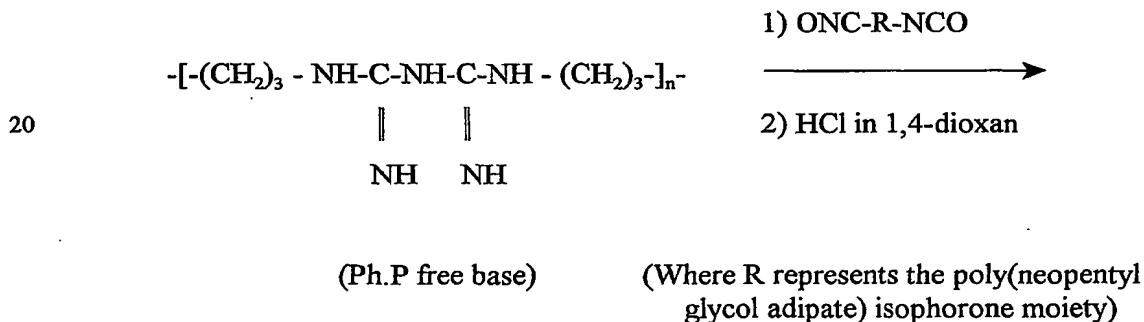
5

Example 3*Polyhexanide/polyisophorone urethane polymer*

1.027g (7.61 x 10⁻⁴ moles) Poly (neopentyl glycol adipate) isophorone diisocyanate terminated (PNGAID, Mn 1350) (Aldrich Chemical Co.) was dissolved in 50ml dichloromethane. 1g of Ph.P free-base (from Example 2) was dissolved in 50ml ethanol and was vigorously stirred. To this polyhexanide was added the above PNGAID solution over a period of 1hr, to form urethane urea bonds. The solution was neutralised with 0.019ml of 4M hydrogen chloride in 1,4 dioxan.

15

Infrared spectrum showed the disappearance of the band at 2265.9cm due to the N=C=O group.

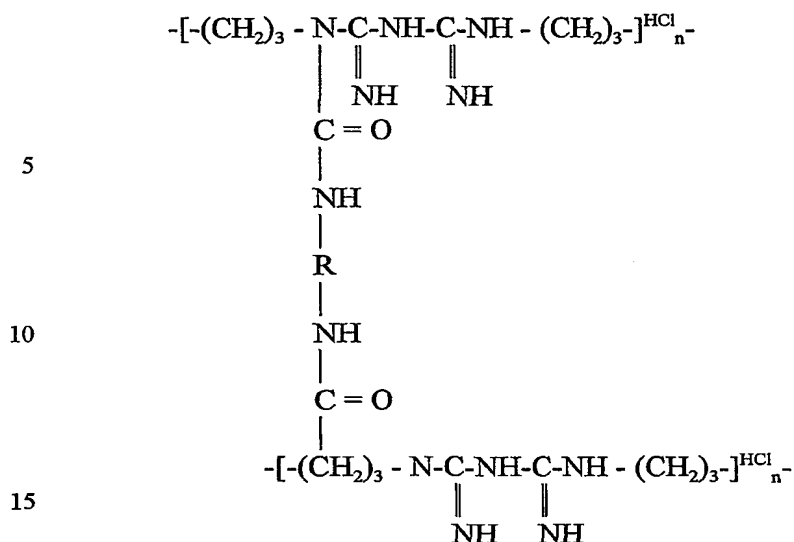


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Example 4*Polyhexanide/silicone copolymer*

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4.158g (3.08×10^{-3} moles) PNGAID was dissolved in 50ml anhydrous dichloromethane and stirred vigorously. 41.58g (1.54×10^{-3} moles) aminopropyl terminated polydimethylsiloxane (APDS) with an average molecular weight of 27,000 (Gelect, Inc) was dissolved in 200ml anhydrous dichloromethane, and was added drop-wise to the
 25 dichloromethane solution of PNGAID over a period of 2hrs. The reaction formed urethane urea bonds between the PNGAID and APDS with the resulting copolymer terminating in isocyanate groups.

The infrared spectrum of the copolymer showed the existence of the band at 2265cm^{-1}
 30 due to the $\text{N}=\text{C}=\text{O}$ group.

To the above PNGAID-APDS copolymer was added allylamine (0.088g, 1.54×10^{-3} moles) dissolved in 50ml anhydrous dichloromethane over a period of 1hr. This resulted in the introduction of one allyl functionality to the PNGAID-APDS copolymer leaving
 35 one reactive isocyanate.

-22-

1g of Ph.P free-base (from Example 2) was dissolved in a mixture containing 40ml ethanol and 10ml dichloromethane. This solution was vigorously stirred and the above copolymer PNGAID-APDS was added dropwise over a period of 2hrs. Infrared showed the disappearance of the $\text{N}=\text{C}=\text{O}$ band at 2265cm^{-1} . The secondary amine of the Ph.P free-base reacted with the isocyanate of the PNGAID-APDS copolymer to form a urethane urea bond.

The resulting solution was neutralised with 0.019ml of 4M hydrogen chloride in 1,4 dioxan.

Example 5

Extruded silicone sheets

The copolymer resulting from Example 4 was dried initially on a rotary evaporator and then dried under vacuum at 50°C for 16hrs. The yield was 47g of polyhexanide/silicone copolymer.

The above polyhexanide/silicone copolymer (47g) was mechanically mixed in with Silastic Q7-4736 Biomedical grade ETR (1Kg) obtained from Dow Corning. After 1hr of mechanical mixing, sheets were extruded and cured at 120°C for 30 minutes.

The high consistency silicone sheets containing polyhexanide moieties had tear strengths, elongation and tensile strength equivalent to those containing no polyhexanide/silicone copolymer.

Example 6

Polyhexanide methacrylate

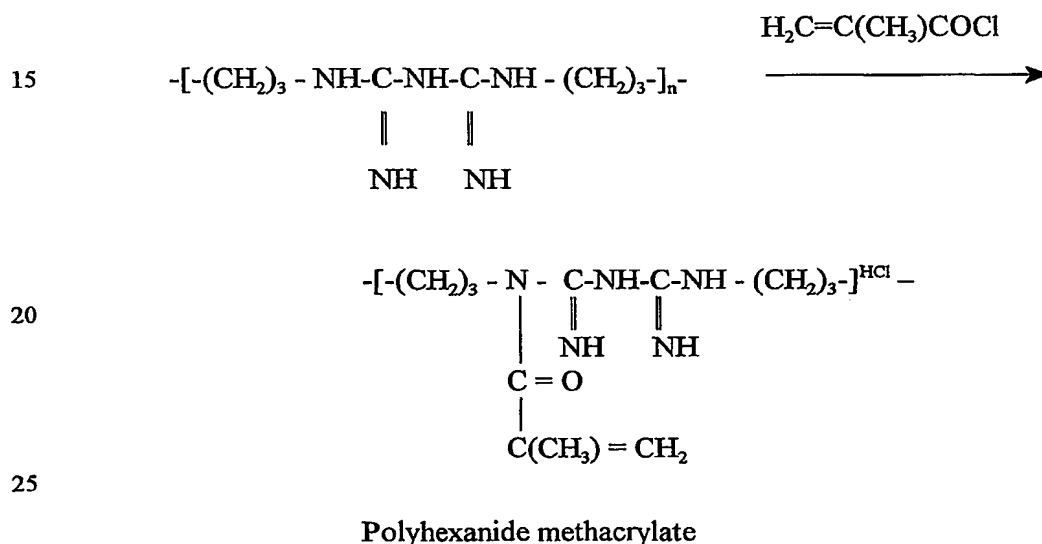
2.25g (0.01027 moles of biguanide hydrochloride groups) of polyhexanide powder (from Example 1) was dissolved in de-ionised water. The number of moles of sodium hydroxide required to neutralise 1 in 12 biguanide hydrochloride groups of polyhexanide is 8.5616 x

-23-

10^{-4} moles (0.03425g NaOH). Sodium hydroxide (0.03425g) was dissolved in 50ml of de-ionised water and added dropwise to the vigorously stirred solution of polyhexanide partial free-base (Ph.P free-base).

- 5 The above Ph.P free-base was dissolved in 60ml anhydrous dimethyl sulfoxide and stirred. 0.0895g (8.5616×10^{-4} moles) methacryloyl chloride was dissolved in 25ml dimethyl sulfoxide solution containing the Ph.P free-base over a period of 1hr.

The above reaction involves the formation of a tertiary amide when the methacryloyl
10 chloride reacts with the free secondary amine of the polyhexanide and the HCl liberated re-forms the hydrochloride of biguanide groups. Accordingly, when $n=12$, there is an introduction of one methacrylate group per polyhexanide polymer chain.



- 500ml of chloroform was then added to the above reaction mixture which precipitated the derivatised polyhexanide from solution. The solution was allowed to stand for 24hrs at
30 3°C and then washed with 3 x 100ml of chloroform and then dried in a vacuum oven at 30°C for 6hrs.

The infrared spectrum showed the disappearance of the bands at 1765 and 1737 cm^{-1} due to strong absorption of C=O unsaturated aliphatic acid chlorides.

5 The band due to tertiary amide (-C(=O)-N<) stretching in the region 1670-1630 is partially obscured by the absorption due to the polyhexanide.

Example 7

Polyhexanide polymer coated tubing

10

20g of polyhexanide methacrylate was synthesised according to Example 6.

A 2 litre, 3-necked reaction vessel equipped with stirrer, thermometer, condenser and nitrogen inlet tube was charged with 1000ml of de-ionised water and placed in a silicone oil bath at 120°C. The solution was stirred gently, and nitrogen was bubbled through the solution (40cm³/min).

140g methoxy polyethyleneglycol 2000 methacrylate (MPEG2000MA) (Inspec) was placed in a 1 litre beaker and dissolved with stirring in 100ml de-ionised water. Then 36g methoxy polyethyleneglycol 350 methacrylate (MPEG350MA) (Inspec) was poured into the MPEG2000MA solution. Then 85g of butyl methacrylate (Aldrich) was poured into the above solution with vigorous stirring.

When the temperature in the 2 litre, 3-necked reaction vessel reached 75°C the above
25 monomer mixture was poured into the reaction vessel and was stirred vigorously.

20g of polyhexanide methacrylate was dissolved in 100ml de-ionised water and was poured into the above reaction vessel containing the monomer mixture. When the temperature inside the reaction vessel reached 80°C, 1g potassium persulphate (dissolved in 60ml of de-ionised water) was added to the reaction vessel.

-25-

After approximately 10mins a white viscous emulsion polymer had formed. The polymer was cooled down to room temperature in a water bath and then poured into a dialysis membrane (MWCO 3,000-4000) and dialysed against 10 litres of water for 48hrs. After 24hrs, the 10 litres of water was replaced with fresh de-ionised water.

5

The polymer was removed from the dialysis membrane and poured into freeze-drying trays and was then freeze-dried for 72hrs.

200g of a dry white powder of the polymer resulted.

10

2g of the above polymer was dissolved in 30ml isopropanol and when fully dissolved to a clear solution, 70ml of tetrahydrofuran was added. Polyvinylchloride (PVC) and polyurethane (PU) tubing were coated with this polymer by dipping the tubing into the above polymer solution and then allowing it to dry for 2hrs. When wetted with water, both the PVC and PU tubings were completely wetted out and were highly lubricious. 10 PVC and 10 PU (length 5cm) tubings were then incubated at 37°C in de-ionised water (100ml) for 72hrs. They were then removed, washed and tested for wetting. Again both sets of tubings were completely wetted out and there was no diminishing in the lubricity. The de-ionised water in which the PVC and PU tubes were incubated was freeze-dried and re-constituted in 3ml de-ionised water and the absorbance of the solution was measured at 250nm. No absorption was detected due to the polyhexanide. A 0.0025% w/v solution of polyhexanide, which was used as the control, had an absorption of 0.35 o.d.

25

Example 8

Chlorhexidine methacrylate

1g (1.9784×10^{-3} moles) chlorhexidine (Aldrich) was dissolved in 100ml anhydrous dichloromethane and stirred vigorously. 0.207g (1.9784×10^{-3} moles)

30

methacryloylchloride was dissolved in 50ml anhydrous dichloromethane and added drop-wise to the chlorhexidine solution over a period of 1hr. Methacryloylchloride reacted with

-26-

the secondary amine of the chlorhexidine forming a tertiary amide. The hydrogen chloride liberated formed one hydrochloride on the chlorhexidine. The reaction was followed by infrared and observed the disappearance of the peaks of methacryloylchloride.

- 5 Chlorhexidine methacrylate monohydrochloride was formed in the above reaction. In order to obtain the dihydrochloride, 0.495ml of 4M hydrogen chloride in 1,4 dioxan was added to the reaction mixture.

The solvent was rotary evaporated off to leave a dry powder of chlorhexidine
10 methacrylate dihydrochloride (CMD).

Example 9

Chlorhexidine methacrylate

- 15 1g (1.9784×10^{-3} moles) chlorhexidine was dissolved in 100ml anhydrous dichloromethane and stirred vigorously. 0.3067g (1.9784×10^{-3} moles) 2-isocyanatoethyl methacrylate (IEM) was dissolved in 50ml anhydrous dichloromethane and added drop-wise to the chlorhexidine solution over a period of 1hr. IEM reacted with the secondary amine of the chlorhexidine to form a urethane urea bond. Infrared
20 showed the disappearance of the isocyanate peak due to IEM.

The above reaction resulted in the formation of chlorhexidine methacrylate. In order to obtain the dihydrochloride, 0.99ml of 4M hydrogen chloride in 1, 4 dioxan was added to the reaction mixture. The solvent was rotary evaporated to yield chlorhexidine
25 methacrylate dihydrochloride.

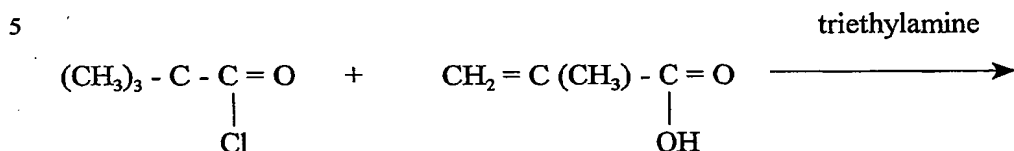
Example 10

Chlorhexidine amide linkage with methacrylic acid

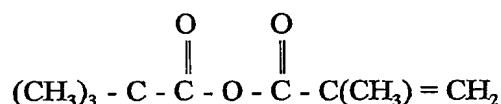
- 30 1g (8.293×10^{-3} moles) trimethylacetyl chloride was dissolved in 50ml anhydrous dichloromethane. To this was solution added 0.839g distilled triethylamine and the

-27-

solution stirred. 0.714g (8.293×10^{-3} moles) methacrylic acid was dissolved in 25ml anhydrous dichloromethane and added drop-wise to the above mixture and stirred for 3hrs at 22°C. This reaction resulted in the formation of a mixed anhydride.



10



The dichloromethane was rotary evaporated leaving a liquid mixed anhydride.

15

1g (1.9784×10^{-3} moles) chlorhexidine was dissolved in 100ml anhydrous dichloromethane and stirred vigorously.

The above mixed anhydride was then reacted on a mole to mole basis with the
 20 chlorhexidine. The number of grams of mixed anhydride required was 0.3363g (1.978×10^{-3} moles). However, to allow for the triethylamine hydrochloride in the mixture, the number of grams required is 0.61g. Accordingly 0.61g of the mixed anhydride mixture was dissolved in 50ml anhydrous dichloromethane and added dropwise to the chlorhexidine solution over a period of 1hr. The methacrylic acid formed a tertiary amide
 25 with the secondary amine of the chlorhexidine and the trimethyl acetic acid formed the counter ion to form chlorhexidine methacrylate mono trimethylacetate. 0.495ml of 4M hydrogen chloride in 1,4 dioxan was added to form the other counter ion. The solution was rotary evaporated to dryness and a dry powder of the product obtained.

30 Chlorhexidine will preferentially react with the methacrylic acid of the mixed anhydride and not the trimethylacetic acid because the latter is sterically hindered.

Example 11*Chlorhexidine methacrylate homopolymer*

10g (0.01978 moles) chlorhexidine was dissolved in 60ml anhydrous dimethylsulfoxide
5 under nitrogen at 40°C (approximately 30mins) in a 3-necked round bottom flask with
overhead stirring. The solution was allowed to cool to room temperature and then 2.81g
glycidyl methacrylate was added, followed by 0.2g triethylamine. The solution was
allowed to react for 4hrs at 60°C with stirring. Then the dihydrochloride salt was formed
by adding 10ml of 4M hydrogen chloride in 1,4 dioxan to give chlorhexidine methacrylate
10 dihydrochloride.

Nitrogen was then bubbled through the solution and the temperature of the solution
allowed to reach 75°C when 0.1g 2,2'-azobis (2,4-dimethylvaleronitrile) was added as
initiator. The solution became highly viscous after 15min polymerisation at 80°C and at
15 30min the reaction was stopped by cooling the solution. The homopolymer of
chlorhexidine was precipitated by adding 500ml de-ionised water. The polymer was
washed several times with water before drying in a vacuum oven at 60°C for 24hrs.

Example 12*Chlorhexidine methacrylate*

Chlorhexidine methacrylate dihydrochloride was made exactly as according to Example
11, except that the solvent used was anhydrous chloroform. The chloroform was rotary
evaporated to leave a dry white powder.

Example 13*Chlorhexidine/polyisophorone urethane polymer*

3g (5.935×10^{-3} moles) chlorhexidine was dissolved in 100ml anhydrous dichloromethane
30 with stirring. 4g (2.963×10^{-3} moles) PNGAID was dissolved in 50ml anhydrous
dichloromethane and added drop-wise to the chlorhexidine solution over a period of 1hr.

When the reaction was complete, infrared showed the absence of the bond at 2265.9cm^{-1} due to $\text{N}=\text{C}=\text{O}$ group of PNGAID. The reaction resulted in forming a urethane urea bond. 2 mole equivalent of chlorhexidine reacted with one mole equivalent of PNGAID.

- 5 Then the dihydrochloride was formed by adding 2.97ml of 4M hydrogen chloride in 1,4 dioxan. Five 5cm long PU tubings were dip-coated with this polymer and allowed to dry for 24hrs. The PU tubing was then placed in de-ionised water (100ml) at 37°C for 72hrs, after which the tubes were removed and the de-ionised water was freeze-dried. 3ml of anhydrous dichloromethane was used to wash the stainless steel tray in which the de-
- 10 ionised water was freeze-dried. A potassium bromide crystal was coated with the washing of dichloromethane and then infrared spectroscopy was conducted. Infrared showed no peak which related to the chlorhexidine or to the PNGAID.

CLAIMS

1 Use of a chemical material containing at least one biguanide group



5 in the preparation of a medicament or medical device for the inhibition of mammalian cell growth.

2 Use of a chemical material according to claim 1 that contains more than one biguanide group.

10

3. Use of a chemical material according to claim 2 wherein the material that contains more than one biguanide group is a polymer.

4 Use of a chemical material according to claim 1 that contains a single
15 biguanide group.

5 Use of a chemical material according to any one of claims 1 to 4 in a composition also containing one or more of an anti-cancer agent, antibiotic agent and a drug.

20 6 Use of a chemical material according to any one of claims 1 to 5 in the
preparation of a medicament which is in a form suitable for direct administration.

7 Use of a chemical material according to claim 6 to release the material through
solution, ablation, leaching, erosion, or cell-mediated erosion, or in the form of a solution,
25 suspension, colloidal solution or suspension, or emulsion.

8 Use of a chemical material according to any one of claims 1 to 5 in the preparation of a medical device by coating the medical device with one or more layers containing the material.

9 Use of a polymeric material incorporating a biguanide compound pendant to the polymer chain, being chemically bound thereto through some but not all of the amine nitrogen atoms of the biguanide compound in the construction of a medical device resistant to the growth of mammalian cells.

5

10 Use of a polymeric material according to claim 9 wherein the pendant biguanide compound is chemically bound to the polymer chain through secondary amine nitrogen atoms of the -NC-C(NH)-NH-C(NH)-NH- biguanide group or groups of the biguanide compound.

10

11 Use of a polymeric material according to claim 9 or claim 10 wherein the biguanide compound is the residue of chlorhexidine or polyhexanide.

12 Use of a polymeric material according to claim 9 or claim 10 wherein the
15 biguanide compound provides residues of both chlorhexidine and polyhexanide

13 Use of a polymeric material according to any of claims 10 to 12 wherein the said chemical binding to secondary amine nitrogen atoms is by means of a substituted urea linkage, or a substituted thiourea linkage, or a N,N-disubstituted amide linkage, or a N,N-
20 disubstituted hemiaminal or aiminal linkage, or a tertiary amine linkage.

14 Use of a polymeric material according to any of claims 9 to 13 wherein the medical device is formed from or coated with the polymeric material incorporating the biguanide compound.

25

15 Use of a polymeric material according to any of claims 9 to 13 wherein the medical device is first formed from or coated with polymeric material which is thereafter chemically bound to some but not all of the nitrogen atoms of the biguanide compound,

30 16 Use of a polymeric material according to any of claims 9 to 13 wherein the medical device is first formed from or coated with polymeric material which is thereafter

chemically bound to the residuum of a non-polymeric compound that has been bound to some but not all of the nitrogen atoms of the biguanide compound.

17 Use of a polymeric material according to any of claims 9 to 16 wherein the medical
5 device is a stent.

18 Use of a polymeric material according to any of claims 9 to 16 wherein the medical
device is a prosthesis, implanted sensor, or implanted capsule that contains cells or
releases a drug.

10

19 Use of a polymeric material according to claim 1 substantially as hereinbefore
described.

20 A method for associating a biguanide with a medical device substantially as
15 described herein.

21 A method of treating, preventing or inhibiting restenosis of a vascular graft
substantially as described herein.

20 22 A device for treating, preventing or inhibiting restenosis of a vascular graft
substantially as described herein.

23 A composition that comprises a chemical material containing at least one
biguanide group

25



in conjunction with one or more of an anti-cancer agent, antibiotic agent and a drug.

24 A composition according to claim 23 wherein the agent or drug comprises
rapamycin, actinomycin or taxol.

30

25 The use of a composition according to claim 23 or 24 for delivery of the agent or drug in the treatment of a mammal, or in the preparation of a medicament for such purpose.

INTERNATIONAL SEARCH REPORT

ional Application No

PCT/GB 02/00672

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K31/155 A61L27/40 A61L27/54 A61L29/12 A61L29/16
A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K A61L A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE CANCERLIT 'Online! XU S H: "In vitro prompt killing by chlorhexidine of human colorectal carcinoma cell lines." retrieved from STN Database accession no. 90305677 XP002200735 abstract & CHUNG-HUA WAI KO TSA CHIH 'CHINESE JOURNAL OF SURGERY!, (1990). VOL. 28, NO. 1 40-2, PP. 62., Hangzhou.</p> <p style="text-align: center;">--- -/--</p>	1,2,4,6, 7



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
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- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

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- *G* document member of the same patent family

Date of the actual completion of the international search

31 May 2002

Date of mailing of the international search report

21/06/2002

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Authorized officer

Villa Riva, A

INTERNATIONAL SEARCH REPORT

ional Application No

PCT/GB 02/00672

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MARIOTTI ANGELO J ET AL: "Chlorhexidine-induced changes to human gingival fibroblast collagen and non-collagen protein production." JOURNAL OF PERIODONTOLOGY, vol. 70, no. 12, December 1999 (1999-12), pages 1443-1448, XP001070990 ISSN: 0022-3492 abstract figure 1 page 1446-1447 "Discussion"	1,2,4,6, 7
X	PITFIELD B ET AL: "Effect of chlorhexidine on gingival epithelial cell growth and migration." JOURNAL OF DENTAL RESEARCH, vol. 72, no. ABSTR. SPEC. ISSUE, 1993, page 336 XP001070981 Joint Meeting of the International Association for Dental Research, the American Association of Dental Research and the Canadian Association of Dental Research; Chicago, Illinois, USA; March 10-14, 1993 ISSN: 0022-0345 abstract	1,2,4,6, 7
X	SHAKESPEARE V ET AL: "Effects of proprietary oral rinses containing chlorhexidine, hexetidine and benzydamine on the proliferation of human buccal epithelial cells in culture." ARCHIVES OF ORAL BIOLOGY. ENGLAND 1988, vol. 33, no. 12, 1988, pages 881-885, XP001070982 ISSN: 0003-9969 abstract figures 1,2 page 883, right-hand column, line 15 - line 27 page 884, left-hand column, line 7 - line 10	1,2,4,6, 7
X	RO 113 302 B (NEACSU ION ;OITA N NICOLAIE (RO); AGRIGORAEI GABRIELA (RO); AGRIG) 30 June 1998 (1998-06-30) abstract column 1, line 41 -column 2, line 1 example 2	1,2,5-7, 23-25
X	US 5 886 048 A (JETHON FRANK ET AL) 23 March 1999 (1999-03-23) abstract column 2, line 5 - line 11 column 2, line 54 - line 56	1-3,6
	-/--	

INTERNATIONAL SEARCH REPORT

tional Application No
PCT/GB 02/00672

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE WPI Section Ch, Week 199320 Derwent Publications Ltd., London, GB; Class A96, AN 1993-164368 XP002200736 & JP 05 097697 A (LION CORP), 20 April 1993 (1993-04-20) abstract</p> <p>---</p>	23,25
X	<p>DATABASE WPI Section Ch, Week 198248 Derwent Publications Ltd., London, GB; Class A96, AN 1982-03411J XP002200737 & JP 57 171913 A (SHOWA YAKUHI KAKO KK), 22 October 1982 (1982-10-22) abstract</p> <p>---</p>	23,25
X	<p>WO 95 04520 A (EMBRO WILLIAM J) 16 February 1995 (1995-02-16) abstract; examples page 1, line 4 - line 5 page 2, line 1 - line 10</p> <p>---</p>	23-25
X	<p>EP 0 490 250 A (BOEHRINGER INGELHEIM INT) 17 June 1992 (1992-06-17) page 2, line 1 - line 3 page 2, line 38 page 6, line 8 - line 12 claims 1,3</p> <p>---</p>	23-25
X	<p>RO 112 577 B (INST POLITEHNIC) 28 November 1997 (1997-11-28) abstract column 1, line 32 - line 35 claim 1; example 1</p> <p>---</p>	23-25
Y	<p>WO 86 02561 A (BROHULT JOHAN; WERNER BJOERN; EDENROTH PER) 9 May 1986 (1986-05-09) abstract claims</p> <p>---</p>	1-25
Y	<p>EP 0 379 269 A (BECTON DICKINSON CO) 25 July 1990 (1990-07-25) the whole document</p> <p>-----</p>	1-25

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

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